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13. ABSTRACT *(Maximum 200 words)*

Cell membranes play a pivotal role in signal transduction and information processing. This is owing to the fact that most physiological activities involve some kind of lipid bilayer-based receptor-ligand contact interactions. There are many outstanding examples such as ion sensing, antigen-antibody binding, and ligand-gated channels, to name a few. One approach to study these interactions in vitro is facilitated by employing artificial bilayer lipid membranes (BLMs). We have focused the efforts on ion and/or molecular selectivity and specificity using newly available self-assembled BLMs on solid support (i.e. s-BLMs), whose enhanced stability greatly aid in research areas of membrane biochemistry, biophysics and cell biology as well as in biosensor designs and molecular devices development. In this report, our current work along with the experiments done in collaboration with others on s-BLMs will be presented.

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Final Report

Specific Aims - The aims of this project are the same as those stated in the original proposal submitted in 1991, namely, the elucidation of mechanisms associated with charge transport, electronic processes, and redox reactions in then newly developed supported bilayer lipid membranes (s-BLMs), and to develop appropriate biosensors using s-BLMs.

Results - Much experimental work has been carried out on the conventional BLM system. This is a natural result of two well known facts: (i) an artificial BLM has been proven to be the closest experimental model of biomembranes, and (ii) in living organisms, biomembranes are essentially the basic structure of Nature's sensors and devices. With s-BLMs, we have shown that lipid bilayers containing compounds such as valinomycin, monensin, gramicidin, iodine, DP-TTF, and TCNQ are sensitive to various ions e.g., H^+ and Na^+. Of particular significance are the sensitivity of s-BLMs containing TCNQ to glucose, and DP-TTF to hydrogen peroxide. To date, accomplishments documented in 17 publications and manuscripts (in press and submitted) include the preparation and characterization of self-assembled bilayer lipid membranes on solid support (s-BLMs), hydrogen peroxide-sensitive electrode based on s-BLMs, alkaline-pretreated aluminum electrode as pH sensors, a review article on the use of BLM systems for biomolecular electronic devices development, immobilization of ferrocene on a s-BLM as amperometric sensor of $Fe(CN)_6^{3-/4-}$ ions, two Ph.D. theses [Tities: Anionic permeability of the liver ER membranes, and Responses of reconstituted olfactory receptors to diethylsulfide derivatives], cyclic voltammetry studies of s-BLMs modified with electron mediators, TCPBQ/TCOBQ modified s-BLMs for pH measurements, TCNQ-modified s-BLMs for glucose detection and s-BLMs on interdigital electrodes prepared by microelectronic techniques. This last mentioned accomplishment demonstrates the potential practical applications of s-BLMs.

In the following paragraphs, a summary of these and other research accomplishments are presented.

Summary of Research Accomplishments

1. **Preparation and Characterization of supported bilayer lipid membranes (s-BLMs).** Bilayer lipid membranes (or lipid bilayers) on solid support have been formed by two consecutive steps: (i) placing a lipid solution in contact with a freshly cut teflon-coated metal wire, and (ii) immersing the lipid layer that has adsorbed onto the metal surface into an aqueous solution. For the best cutting of metal wires, a miniature guillotine has been used, where the sharp knife is moved vertically onto the wire placed on a flat surface and immersed in a lipid solution. Typically we used either a 1% glycerol dioleate in squalene or 1% phospholipid (lecithin) in n-decane. Compounds of interest for incorporation into lipid bilayer were dissolved in the lipid solution prior to cutting. As evidenced by monitored electrical parameters (capacitance, resistance, potential, and/or current/voltage characteristics), a self-assembled BLM was formed within ten minutes or so.

2. **Immobilization of ferrocene on s-BLMs: an amperometric sensor of** ferri/ferro cyanide ions. The redox reactions for a solid supported BLM containing vinyl-ferrocene as an electron mediator have been investigated using

cyclic voltammetry. The results have shown that (i) ferrocene can be very easily immobilized in the lipid bilayer on the surface of a metallic wire (s-BLM) system. This demonstrates that the s-BLM system offers a novel approach to the electrode modification by simple way of immobilization of compounds within BLM, and (ii) ferrocene in a bilayer lipid membrane increases about two orders of magnitude a potassium ferri/ferro-cyanide ion sensitivity of the platinum electrode.

3. **Hydrogen peroxide-sensitive s-BLMs.** As proposed in our grant application, the insertion of appropriate active molecules (modifiers) into the matrix of the lipid bilayer should be able to impart the functional characteristics of s-BLMs. We chose TCNQ (tetracyanoquinodimethane) and DP-TFF (dipyridyl-tetrathiafulvalene) as modifiers because of their properties as typical electron and donnor molecules, respectively. It was found that DP-TTF could improve not only the stability but also increased the range of s-BLM's sensitivity to hydrogen peroxide. In contrast, TCNQ-containing s-BLMs did not show much responses to H_2O_2. This was not entirely unexpected since TCNQ should behave as an electron acceptor.

4. **Modified s-BLMs as pH sensors.** Of all the ions crucial to the functioning of cellular processes is the hydrogen ion (H^+) which plays the leading role in enzyme catalysis and membrane transport. Thus, it is not surprising that, the measurement of pH is of the utmost importance. Currently, the pH glass electrode is routinely used in chemical and clinical laboratories. However, the large size and fragility of pH glass electrodes preclude their use in many situations such as in vivo cell studies and in monitoring membrane boundary potentials. For example, the hydrolysis of membrane lipids by phospholipid enzymes (lipases A and C) changes the boundary potential of BLM (or cell membrane) as a result of local pH change. These facts suggest that s-BLMs can be used as a pH probe in membrane biophysical research and in biomedical fields where the conventional glass electrode presents many difficulties. To test our concept, we incorporated a number of quinonoid compounds (chloranils) into s-BLMs. We have found that, indeed, s-BLMs containing either TCOBQ or TCPBQ responded to pH changes with nearly theoretical slope (55 +/- 3 mV). This new pH-sensitive s-BLM offers prospects for ligand-selective probe development using microelectronics technologies.

5. **Alkaline pre-treated Al as pH sensors.** In view of the paramount importance of pH monitoring in membrane research and in biomedical fields, some experiments were also carried out using ultrathin wires. We have found that certain specially annealed Al wires can function very well as pH sensors. Briefly, the potential-pH relations of Al wire electrode were studied in 0.1 M KCl of buffered solutions. Linear pH responses having a slope of 90 +/- 4 mV/pH over the pH range 3-14 were obtained. The results have been explained in terms of the hydration of Al oxides and the formation of Al hydrides. The probe diameter (tip area) and ionic strength of the bathing solution have little effects on pH responses. The reproducibility of the short-term stability as well as the probe lifetime were also investigated. On the basis of the findings, this type of Al-wire probe opens the way for a host of s-BLM experiments such as the mechanisms of charge carrier generation, transport, redox reactions and energy transduction pertinent to membrane bioenergetics as detailed in the original proposal.

6. **S-BLMs on interdigital electrodes by microelectronic techniques**

The results of our recent research have confirmed a broad spectrum of possible areas of s-BLM applications. All of these possibilities are based upon

the fact that a lipid bilayer structure can be deposited on a solid substrate. This novel manner of lipid bilayer formation overcomes two basic obstacles in the way of the practical utilization of the BLM structure, namely: (i) its stability and (ii) its compatibility with a standard microelectronic technology. As has been demonstrated by us and others, the solid supported BLM systems (s-BLMs) not only possesses advantages of a conventional BLM structure but additionally gain the new important properties such as (a) long-term stability, (b) an anisotropic, highly ordered, yet very dynamic liquid-like structure, (c) two asymmetric interfaces, and (d) this type of probes are predestined for microelectronic fabrication. On this last mentioned property, we extended the experiment described in Item 4 above to the interdigital structures (IDS). IDS are finger-like electrodes made by microelectronic technologies and used in micro-chip applications. By forming s-BLMs on IDS made of platinum with a window of 0.5 x 0.5 mm, we obtained the following interesting results. First, when an IDS coated with a BLM formed from asolectin, it responded to pH changes with 15 +/- 2 mV slope. The conductance of s-BLMs on IDS was about 50 times higher than the usual s-BLMs. Second, when an IDS coated BLM formed from asolectin plus TCOBQ (or TCPBQ), the pH response was linear with a 50 +/- 1 mV slope. This very interesting finding suggests that (i) the lipid bilayer, the fundamental structure of all biomembranes, can be attached to an IDS with responses not unlike those found in s-BLM, (ii) this type of structure (i.e., s-BLM on interdigital electrodes) can be used to investigate ligand-receptor contact interactions, and (iii) s-BLMs on IDS can be manufactured using microelectronic technologies which already exist without the explicit need of special modification. We consider this finding as a major 'breakthrough' in biosensor development and will be pursued by us in the coming years.

Research plans for the requested support period (1994-1997) are as follows: 1) Continuing current experiments in progress; this entails cyclic voltammetry studies of s-BLMs modified with a host of organic compounds and redox proteins, (2) investigation of s-BLM based biosensors using piezoelectric quartz crystals, and (3) investigation of s-BLMs on interdigital electrodes prepared by microelectronic techniques. Focus will be placed on self-assembled lipid bilayers on solid support as an advanced material or as a 'smart' and nanostructured system. In particular, we have succeeded in forming s-BLMs on interdigital structures (IDS) made by microelectronic techniques. This very interesting finding opens the way for investigating ligand-receptor contact interactions; these are necessary first steps towards the development of biosensors for practical applications. These s-BLMs will be studied using various **in situ** techniques developed recently in our laboratory.

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